Antimalarial Compounds from *Grewia bilamellata*[⊥]

Cuiying Ma,[†] Hong Jie Zhang,[†] Ghee Teng Tan,[†] Nguyen Van Hung,[‡] Nguyen Manh Cuong,[§] D. Doel Soejarto,[†] and Harry H. S. Fong*,[†]

Program for Collaborative Research in Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, Institute of Chemistry, Vietnamese Academy of Science and Technology, Cau Giay, Hanoi, Vietnam, and Cuc Phuong National Park, Nho Quan District, Ninh Binh Province, Vietnam

Received August 22, 2005

Bioassay-directed fractionation led to the isolation of 12 compounds from a sample of the dried leaves, twigs, and stems of *Grewia bilamellata*. Five of the isolates, 3α,20-lupandiol (**1**), grewin (**2**), nitidanin (**4**), 2α,3β-dihydroxy-olean-12-en-28-oic acid (**5**), and 2,6-dimethoxy-1-acetonylquinol (**6**), showed varying degrees of in vitro antimalarial activity against *Plasmodium falciparum*, but were devoid of significant cytotoxicity to the human oral epidermoid KB cancer cell line. Of the 12 isolates, compounds **1**, **2**, and **3** (bilagrewin) were determined to be a new triterpene, a new coumarinolignan, and a new neolignan, respectively. Other known compounds isolated in this study were 8-*O*-4′ neolignan guaiacylglycerol-β-coniferyl ether isomers (*threo* and *erythro*), cleomiscosin D, icariol A_2, ciwujiatone, and daucosterol. The structures of **1**−**3** were elucidated and identified on the basis of spectroscopic data including 1D and 2D NMR analysis.

Malaria is a major threat to global health, with about 40% of the world's population being exposed to this infection.[1] Annually, 300−500 million cases of *Plasmodium falciparum* malarial infections are reported worldwide, with a mortality rate of 1.5−3 million.[1] Our International Cooperative Biodiversity Group (ICBG) research project,[2] involving the collaboration of institutions in Vietnam, Laos, and the United States, offers an opportunity to search for potential antimalarial compounds from plants. We have previously reported on the isolation of active antimalarial natural products from *Rhaphidophora decursiva* Scott (Araceae), *Ficus fistulosa* Reinw. ex Bl. (Moraceae), and *Nauclea orientalis* (L). (Rubiaceae).[3−6] *Grewia bilamellata* Gagnep. (Tiliaceae) was found to be a promising lead in an anti-*Plasmodium falciparum* screening study. A literature search revealed no prior phytochemical studies on this plant. Antimalarial bioassay-directed fractionation of the $CHCl_3$-soluble fraction of the MeOH extract prepared from a sample of the combined leaves, twigs, and stems of *G. bilamellata* led to the isolation of eight lignans including two coumarinolignans, four neolignans, two Haworth lignans, two triterpenes, one quinol derivative, and one sterol glucoside. Bioassay results indicated that the isolates 3α,20-lupandiol (**1**), grewin (**2**), nitidanin (**4**), 2α,3β-dihydroxy-olean-12-en-28-oic acid (**5**), and 2,6-dimethoxy-1-acetonylquinol (**6**) possess in vitro antimalarial activity against the D6 and W2 clones of *P. falciparum*. The present paper describes the isolation, structural elucidation, and biological evaluation of the compounds isolated from this plant.

Results and Discussion

A sample composed of the leaves, twigs, and stems of *G. bilamellata* was extracted exhaustively with MeOH, which was concentrated and partitioned sequentially with petroleum ether, $CHCl_3$, and EtOAc. The $CHCl_3$ fraction showed antimalarial activity with IC_{50} values of 2.2 and 1.7 μg/mL to the D6 and W2 clones of *P. falciparum*, respectively. Insignificant cytotoxicity was observed against human oral epidermoid carcinoma cells (KB) (ED_{50} > 20 μg/mL). Consequently, the $CHCl_3$ extract was subjected to antimalarial assay-guided fractionation through a series of separations by silica gel column, Sephadex LH-20 column, C_{18} reverse-phase column, preparative HPLC, and preparative TLC chromatography, leading to the isolation of 12 compounds, five of which were found to be biologically active (**1**, **2**, and **4**−**6**) and three are new isolates (**1**−**3**) being reported for the first time from nature.

3α,20-Lupandiol (**1**) was obtained as microcrystals. Its molecular formula, $C_{30}H_{52}O_2$, was determined on the basis of the HRFABMS at *m*/*z* 467.3902 $[M + Na]^+$ (calcd 467.3865) and supported by the ^{13}C NMR and DEPT spectroscopic data. The IR spectrum showed the presence of OH (3311 cm^{-1}) groups. Analysis of its ^1H, ^{13}C, and DEPT NMR spectra indicated that compound **1** contains 30 carbons, including eight methyls, 10 methylenes, six methines, and six quaternary, but no olefinic, carbons. On the basis of the above analysis, compound **1** was recognized as being a triterpene. On comparing its ^{13}C NMR data with those reported in the literature for triterpenes, the chemical shifts of C-1 through C-10, and C-23 through C-26, were the same as those reported for hopan-3α,22 diol,[7] and the chemical shifts of C-11 through C-22 and C27 through C-30 were the same as those found in 3β,20-lupandiol.[8,9] In the ^1H NMR spectrum, a signal at δ 3.39 (1H, brs, H-3) confirmed the presence of a OH-3α moiety.[7] Analysis of the COSY, HMQC, and HMBC spectra led to the structure 3α,20-lupandiol being proposed for **1**, which is a new natural triterpene.

Grewin (**2**) was obtained as a white amorphous powder. Its molecular formula, $C_{19}H_{16}O_8$, was determined on the basis of the positive HRESIMS at *m*/*z* 395.0814 $[M + Na]^+$ (calcd 395.0743) and supported by the ^1H, ^{13}C, and DEPT NMR data. The IR spectrum showed the presence of hydroxyl (3303 cm^{-1}) and carbonyl (1741 cm^{-1}) groups. The double-bond signals at δ_H 7.64 (1H, d, J = 9.6 Hz, H-4) and δ_H 6.36 (1H, d, J = 9.5 Hz, H-3) and the two singlets at δ_H 7.08 (1H, s, H-8) and δ_H 7.18 (1H, s, H-5) in the ^1H NMR spectrum, as well as the signal at δ_C 161.5 (C-2) in the ^{13}C NMR spectrum, indicated the presence of a 6,7-dioxygenated coumarin group.[10] A singlet at δ_H 3.79 (3H, OCH_3) and *meta*-coupled doublets (J = 1.9 Hz) at δ_H 7.35 (1H, H-6′) and δ_H 7.01 (1H, H-2′) could be assigned to an unsymmetrical 3′-methoxyl-4′,5′-dioxophenyl group. A three-carbon sequence, CH-(O)CH(O)CH_2OH (C-7′, C-8′, C-9′), was deduced by the presence of a doublet at δ_H 5.50 (1H, d, J = 8.0 Hz, H-7′), a multiplet at δ_H 4.46 (1H, m, H-8′), and geminally coupled methylene protons at δ_H 4.21 (1H, bd, J = 12.3 Hz, H-9′) and δ_H 3.98 (1H, bd, J = 12.3 Hz, H-9′), as well as the presence of the carbon signals at δ_C 78.6

[⊥] Dedicated to Dr. Norman R. Farnsworth of the University of Illinois at Chicago for his pioneering work on bioactive natural products.

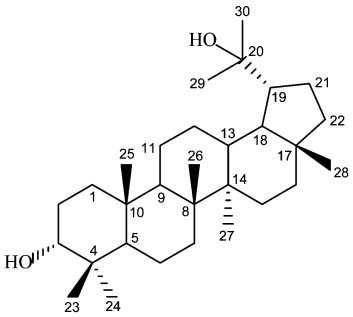
* To whom correspondence should be addressed. Tel: +1-(312) 996-5972. Fax: +1-(312) 996-7107. E-mail: hfong@uic.edu.

[†] University of Illinois at Chicago.

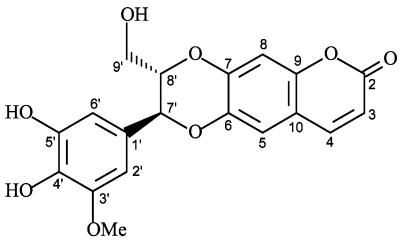
[‡] Vietnamese Academy of Science and Technology.

[§] Cuc Phuong National Park.

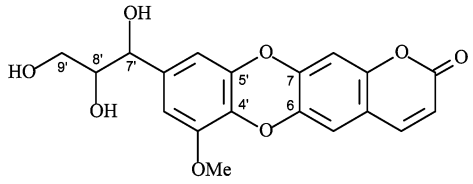
10.1021/np050313d CCC: $33.50
Published on Web 01/05/2006



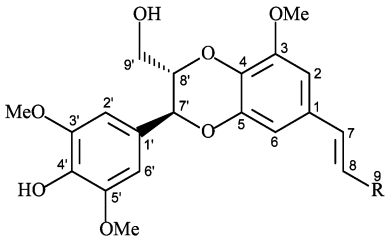
1



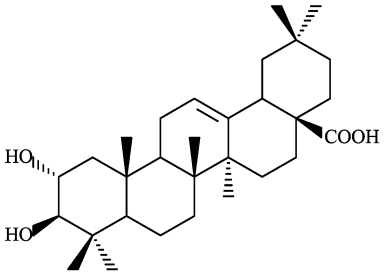
2



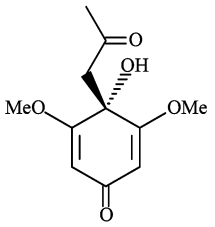
2a



3 R = CHO
4 R = CH_2OH



5



6

(C-7′), δ_C 80.2 (C-8′), and δ_C 61.9 (C-9′). The $^1H-^1H$ COSY and HMBC spectral data (Figure 1) confirmed the presence of these groups. The presence of HMBC long-range correlations between the proton signal at δ_H 5.50 (H-7′) and the carbon signals at δ_C 104.2 (C-2′) and δ_C 110.7 (C-6′) suggested that the three-carbon sequence was attached to the 3′-methoxy-4′,5′-dioxophenyl group. According to the molecular formula of **2**, the double-bond equivalent should be 12, which indicated the presence of an additional ring besides the coumarin ring and the 3′-methoxy-4′,5′-dioxophenyl ring in the structure. On the basis of the above data, the additional ring was elucidated to be a (1,4)-dioxane ring formed

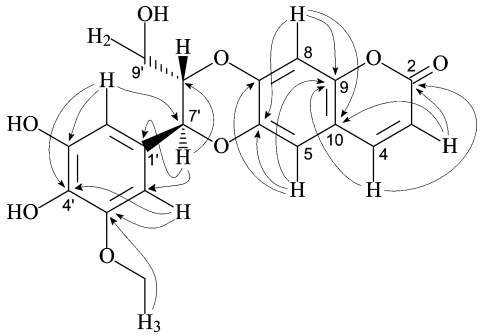


Figure 1. Selected HMBC correlations of compound **2**.

through a connection either between the coumarin group and the $CH(O)CH(O)CH_2OH$ group or between the coumarin group and the 3′-methoxyl-4′,5′-dioxophenyl group, resulting in two possible structures (**2** and **2a**). In the case of **2a**, the coupling constant between H-7′ and H-8′ would be 6 Hz, with the ^{13}C NMR chemical shifts of C-7′, C-8′, and C-9′ approximately 75, 78, and 64 ppm, respectively, for a 7′*R**,8′*R** configured structure and 76, 77, and 64 ppm, respectively, for a 7′*R**,8′*S** configuration.[11] However, the NMR spectra of the isolate revealed the coupling constant between H-7′ and H-8′ to be 8 Hz and the ^{13}C NMR chemical shifts of C-7′, C-8′, and C-9′ to be 78.6, 80.2, and 61.9 ppm, respectively, which are closely comparable to the chemical shifts of the corresponding carbons in nitidanin (**4**)[12] (Table 1). The large coupling constant ($J = 8$ Hz) in **2** could be caused by the inflexible *trans* stereochemistry between H-7′ and H-8′. Thus, grewin was determined to be 2*S**-(3,4-dihydroxy-5-methoxyphenyl)-3*R**-hydroxymethyl-2,3-dihydro-1,4,5-trioxa-anthracen-6-one, which is a new coumarinolignan, and the structure was assigned as **2**.

Bilagrewin (**3**) was obtained as a yellow amorphous powder. Its molecular formula, $C_{21}H_{22}O_8$, was based on the positive HRESIMS at *m*/*z* 425.1206 $[M + Na]^+$ (calcd 425.1212) and supported by the ^1H, ^{13}C, and DEPT NMR spectra. The IR spectrum showed the presence of OH (3343 cm^{-1}) and α,*β*-unsaturated aldehyde (1689 cm^{-1}) groups. A singlet at δ_H 3.83 (3H, OCH_3) and *meta*-coupled doublets ($J = 1.9$ Hz) at δ_H 7.04 (1H, H-2) and 7.21 (1H, H-6) in the ^1H NMR spectrum were assigned to an unsymmetrical 3-methoxy-4,5-dioxophenyl group. The presence of a propenal group was deduced by the observation of ^1H NMR signals at δ_H 7.53 (1H, d, $J = 15.8$ Hz, H-7), 6.96 (1H, dd, $J = 15.7$, 7.8 Hz, H-8), and 9.84 (1H, d, $J = 7.7$ Hz, H-9), together with the ^{13}C NMR signal at δ_C 193.6 (C-9). Analysis of the $^1H-^1H$ COSY and HMBC (Figure 2) spectral data confirmed the presence of the above-mentioned groups. The fact that the signal at δ_H 7.53 (H-7) had long-range correlations with the resonances at δ_C 104.9 (C-2) and δ_C 111.7 (C-6) suggested that the propenal group is attached to the unsymmetrical 3-methoxy-4,5-dioxophenyl group. In the ^1H NMR spectrum of **3**, the appearance of the singlet at δ_H 3.78 (6H, OCH_3) and a singlet at δ_H 7.19 (2H, H-2′ and H-6′) indicated the presence of a symmetrical 4′-hydroxy-3′,5′-dimethoxyphenyl group. A three-carbon sequence, $CH(O)CH(O)CH_2OH$ (C-7′, C-8′, C-9′), was also apparent in **3** due to the presence of a doublet at δ_H 5.52 (1H, d, $J = 8.0$ Hz, H-7′), a multiplet at δ_H 4.43 (1H, m, H-8′), and two geminally coupled methylene protons at δ_H 4.28 (1H, bd, $J = 12.6$ Hz, H-9′) and δ_H 3.88 (1H, m, H-9′), as well as the presence of signals at δ_C 77.3 (C-7′), δ_C 80.3 (C-8′), and δ_C 61.2 (C-9′). Analysis of the HMBC and $^1H-^1H$ COSY spectra confirmed the presence of these groups. The HMBC long-range correlations between the signals at δ_H 5.52 (H-7′) and δ_C 106.2 (C-2′, 6′) indicated that the three-carbon unit between C-7′ through C-9′ was attached to the symmetrical 4′-hydroxy-3′,5′-dimethoxyphenyl group. When the NMR data (Table 1) of **3** were compared to those of nitidanin (**4**),[12] the chemical shifts of the three-carbon sequence and the symmetrical 4′-hydroxy-3′,5′-dimethoxyphenyl group in these two compounds were almost the same. Thus, bilagrewin was determined to be 3-[3*S**-(4-

Table 1. ^{1}H, ^{13}C NMR Data of Compounds **2**−**4** (300, 75 MHz for **2** and **4**; 400, 100 MHz for **3**; pyridine-d_5, δ in ppm, J in Hz)

	2		**3**		**4**	
no.	C	H	C	H	C	H
1			127.0		130.9	
2	161.5		104.9	7.04 (1H, d, J = 1.8)	103.7	6.92 (1H, d, J = 1.9)
3	114.7	6.36 (1H, d, J = 9.5)	150.5		149.8	
4	144.1	7.64 (1H, d, J = 9.6)	137.4		134.5	
5	115.3	7.18 (1H, s)	145.5		145.9	
6	142.1		111.7	7.21 (1H, d, J = 1.9)	109.1	7.09 (1H, d, J = 1.9)
7	148.6		153.3	7.53 (1H, d, J = 15.8)	130.0	6.93 (1H, d, J = 15.4)
8	105.4	7.08 (1H, s)	127.7	6.96 (1H, dd, J = 15.7, 7.8)	130.5	6.65 (1H, dt, J = 15.8, 4.9)
9	150.1		193.6	9.84 (1H, d, J = 7.7)	63.6	4.61 (2H, m)
10	113.8					
1′	127.6		127.0		128.7	
2′	104.2	7.01 (1H, d, J = 1.9)	106.2	7.19 (1H, d, J = 1.9)	106.6	7.16 (1H, d, J = 1.9)
3′	150.2		149.4		149.8	
4′	137.5		138.4		138.7	
5′	148.9		149.4		149.8	
6′	110.7	7.35 (1H, d, J = 1.9)	106.2	7.19 (1H, d, J = 1.9)	106.6	7.16 (1H, d, J = 1.9)
7′	78.6	5.50 (1H, d, J = 8.0)	77.3	5.52 (1H, d, J = 8.0)	77.8	5.49 (1H, d, J = 7.9)
8′	80.2	4.46 (1H, m)	80.3	4.43 (1H, m)	80.4	4.36 (1H, m)
9′	61.9	4.21 (1H, bd, J = 12.3)	61.2	4.28 (1H, bd, J = 12.6)	61.8	4.26 (1H, bd, J = 12.3)
		3.98 (1H, bd, J = 12.3)		3.88 (1H, m)		4.06 (1H, bd, J = 12.3)
OMe	56.7	3.79 (3H, s)	56.4	3.78 (6H, s)	56.9	3.87 (6H, s)
OMe			56.0	3.83 (3H, s)	56.4	3.95 (3H, s)

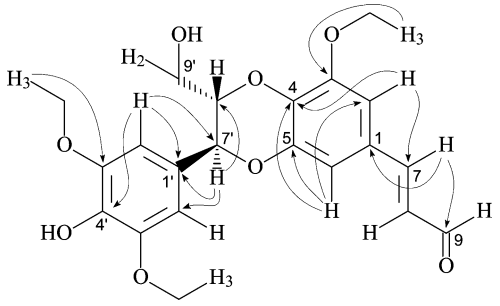


Figure 2. Selected HMBC correlations of compound **3**.

hydroxy-3,5-dimethoxyphenyl)-2*R**-hydroxymethyl-8-methoxy-2,3-dihydrobenzo[1,4]dioxin-6-yl]propenal, which is a new neolignan, and the structure was assigned as **3**.

The known compounds isolated in this study were identified as nitidanin (**4**),[12] 2α,3β-dihydroxyolean-12-en-28-oic acid (**5**),[13] 2,6-dimethoxy-1-acetonylquinol (**6**),[14] a mixture of 8-*O*-4′ neolignan-guaiacylglycerol-β-coniferyl-ether isomers (*threo* and *erythro*),[15] cleomiscosin D,[16] icariol A_2,[17] ciwujiatone,[18] and daucosterol[19] by comparison of their physical and spectroscopic data to those published in the literature.

All isolates were tested for antimalarial activity on cultures of *P. falciparum* clones D6 and W2. Biological evaluation showed that five of the isolates (**1**, **2**, **4**−**6**) possess varying degrees of antimalarial activity without significant cytotoxicity. Compound **2**, a new coumarinolignan, displayed weakly antimalarial activity with no discernible cytotoxicity (ED_{50} >107.5 μM) (Table 2) and selectivity indices of >9.6 and >19.7, for clones D6 and W2, respectively. In light of these favorable in vitro selectivity index values of this compound, in vivo studies involving *Plasmodium berghei* in mice are under consideration. Compound **4**, a neolignan, displayed weak antimalarial activity with minimal cytotoxicity (ED_{50} >99.0 μM) (Table 2). Neolignans with antimalarial activity were previously reported, but were highly cytotoxic.[3] The two triterpenoids (**1**, **5**) also displayed weak antimalarial activity. Compound **6**, a quinol derivative, displayed weak antimalarial activity also (Table 2).

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. IR spectra were obtained on a JASCO FT/IR-410 spectrometer, equipped with a Specac Silver Gate ATR system, by applying a film on a germanium crystal. 1D and 2D NMR spectra were recorded using Bruker AVANCE-400 and DPX-300 MHz NMR spectrometers. Chemical shifts (δ) are expressed in ppm with reference to TMS or the solvent signals. ESIMS and HRESIMS were recorded on a Micromass QTOF-2 spectrometer. FABMS and HRFABMS were recorded on a JEOL GC-mate II spectrometer. Reversed-phase HPLC was carried out on a Waters 600E delivery system pump, equipped with a Waters 996 photodiode array detector and a Phenomenex C_{18} column (250 × 50 mm, 10 μm, 120 Å). Silica gel (200−400 mesh, Natland International Corporation), C_{18} reversed-phase silica gel (40−63 μm, EM Science), and Sephadex LH-20 (25−100 μm, Sigma) were used for column chromatography. TLC analysis was performed on glass-backed plates coated with 0.25 mm layers of silica gel 60 F254 (Merck, Darmstadt, Germany).

Plant Material. The initial sample composed of dried leaves, twigs, and stems (SVA-0849) of *Grewia bilamellata* was collected from a limestone forest at Cuc Phuong National Park, Vietnam, in December 1999, and was documented by a set of voucher herbarium specimens (*Soejarto et al. 11333*); a duplicate specimen has been deposited at each of the herbaria located at Cuc Phuong National Park (*CPNP*), Vietnamese Academy of Science and Technology (formerly National Center for Science and Technology) (*HN*; Hanoi), and the Field Museum (*F*; Chicago). A larger sample (SVA-0849, 6.2 kg, documented by the voucher specimen *Nguyen Manh Cuong et al. 1670*) was re-collected from the same site at Cuc Phuong in June 2002 for the present study.

Antimalarial Assays. Antimalarial assays were conducted with cultured *Plasmodium falciparum* clones (W2 and D6) as described previously.[3] Concentrations of test compounds and positive controls that inhibited parasite-specific [^{3}H]hypoxanthine incorporation by 50% (IC_{50}) values were determined using nonlinear regression analysis. The pure compounds with IC_{50} < 10 μg/mL were considered to have antimalarial activity. Zero-drug controls defined 100% incorporation.

Cytotoxicity Assays. Evaluation of the cytotoxic activity of the $CHCl_3$ fraction and all pure compounds against the human oral epidermoid carcinoma cell line (KB) was performed using the sulforhodamine B staining procedure, as described earlier.[3] The ED_{50} values were calculated using nonlinear regression analysis of percent survival versus compound concentration.

Extraction and Isolation. The dried, milled sample (combined leaves, twigs, and stems, 6.2 kg) of *G. bilamellata* was extracted with MeOH to yield 257 g of extract, which was redissolved in 90% H_2O−MeOH and partitioned sequentially with petroleum ether, $CHCl_3$, and EtOAc to yield petroleum ether (19.6 g), $CHCl_3$ (13.3 g), EtOAc (3.4 g), and H_2O (88 g) soluble fractions. Bioassay located the antimalarial

Table 2. Antimalarial Activities of Compounds **1**, **2**, and **4**−**6** against the D6 and W2 Clones of *Plasmodium falciparum*

	cytotoxicity	D6		W2	
extract/compound	ED_{50} (KB) (μM)[a]	IC_{50} (μM)	SI[b]	IC_{50} (μM)	SI[b]
$CHCl_3$ extract[c]	>20	2.3 ± 0.13	>8.8	1.7 ± 0.12	>11.5
1	>90.0	19.8 ± 0.25	>4.5	19.1 ± 1.46	>4.7
2	>107.5	11.2 ± 1.27	>9.6	5.5 ± 0.44	>19.7
4	>99.0	21.2 ± 0.42	>4.6	18.4 ± 1.57	>5.4
5	51.5	21.1 ± 0.27	2.4	8.6 ± 0.34	5.9
6	169.0	42.2 ± 0.97	4.0	23.0 ± 1.40	7.3
chloroquine[d]	30.8	0.0095 ± 0.00097	3245	0.27 ± 0.021	113

[a] An ED_{50} value of ≥ 50 μM is considered noncytotoxic. [b] SI = selectivity index = ED_{50} (KB)/IC_{50} (*P. falciparum*). [c] The ED_{50} and IC_{50} values of the $CHCl_3$ extract are expressed in μg/mL. [d] Positive control.

activity in the $CHCl_3$ fraction with IC_{50} values of 2.3 and 1.7 μg/mL against the D6 and W2 clones, respectively (Table 2). The $CHCl_3$ fraction, therefore, was subsequently chromatographed over a silica gel column (200 g) and eluted with gradient mixtures of petroleum ether−acetone of increasing polarities to afford 33 fractions (F01−F33). The combined fractions F07−F09 and F26−F30 showed strong antimalarial activity with 100% inhibition of plasmodial (W2) growth at a concentration of 10 μg/mL.

Fractions F07−F09 and F26−F30 were separated further, using a Sephadex LH-20 (250 g) columns eluted with $CHCl_3$−MeOH (1:1). From the fractions F07−F09 (878 mg) active fractions F37−F38 were obtained, and each inhibited clone W2 growth by >90% at 10 μg/mL. Preparative HPLC separation of the pooled F37−F38 (618 mg) on a Phenomenex C_{18} column, developed with MeOH−H_2O (94:6), led to the isolation of 3α,20-lupandiol (**1**, 6 mg). From pooled fractions F26−F30 (3130 mg), six active fractions (F51−F52, F58−F61) were obtained. These fractions inhibited clone W2 growth by >90% at 10 μg/mL. Subsequently, fractions F51 and F52 were pooled (648 mg) and were further separated using a silica gel column, eluting with a $CHCl_3$−MeOH gradient to afford active fractions F101−F103. Active fraction F101 (73 mg) was further chromatographed on a silica gel column eluted with a gradient $CHCl_3$−acetone solvent system to afford fraction F110 (12.6 mg), which was further chromatographed on a RP-18 column (eluting with MeOH−H_2O, 1:1), leading to the isolation of 2,6-dimethoxy-1-acetonylquinol (**6**, 7 mg). Fractions F102 and F103 were pooled (93 mg) and chromatographed on a RP-18 column, eluting with MeOH−H_2O (7:3), to yield 2α,3β-dihydroxyolean-12-enoic acid (**5**, 5 mg). Combined fractions F58 and F59 (539 mg) were further separated on a silica gel column, using a gradient of petroleum ether−acetone for elution, to afford active fractions F74 and F75. Fraction F74 (54.5 mg) was further chromatographed on a RP-18 column and developed with MeOH−H_2O (4:6), leading to the isolation of icariol A_2 (6 mg). From fraction F75 (28.0 mg), a crude crystalline material was obtained and recrystallized in MeOH−saturated $CHCl_3$ to afford daucosterol (20 mg). Fraction F60 (471 mg) was chromatographed on a silica gel column and eluted with a CH_2Cl_2−MeOH gradient to afford fractions F62−F67. Fraction F62 (13 mg) was further separated using RP-18 preparative TLC [MeOH−H_2O (6:4)] followed by silica gel preparative TLC [$CHCl_3$−MeOH (9.4:0.6)] to yield cleomiscosin D (3.1 mg) and bilagrewin (**3**, 2.2 mg). From fraction F63 (47.1 mg), nitidanin (**4**, 15 mg) was obtained as a colorless crystal. Fraction F65 (41.1 mg) was further separated using RP-18 preparative TLC [$CHCl_3$−MeOH (6:4)], followed by silica gel preparative TLC [CH_2Cl_2−MeOH (9.2:0.8)] to yield the isomers of 8-*O*-4′-neolignan-guaiacylglycerol-β-coniferyl-ether (*threo* and *erythro* mixture). Fraction F67 (75.8 mg) was subjected to preparative HPLC separation (eluting with CH_3CN−H_2O, 22:78) to yield ciwijatone (3 mg). A colorless crystalline material was obtained from fraction F61 by directed crystallization from MeOH−saturated $CHCl_3$ and further purified by silica gel preparative TLC [CH_2Cl_2−MeOH (9.2:0.8)] to afford grewin (**2**, 6 mg).

3α,20-Lupandiol (1): microcrystals ($CHCl_3$−MeOH, 1:1); mp 162−163 °C; $[\alpha]^{23}_D$ −9.6 (*c* 0.27, CH_2Cl_2); IR (film) ν_{max} 3311, 2929 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.39 (1H, brs, H-3), 1.22 (3H, s, H-30), 1.12 (3H, s, H-29), 1.06 (3H, s, H-26), 0.97 (3H, s, H-27), 0.94 (3H, s, H-23), 0.85 (3H, s, H-25), 0.83 (3H, s, H-24), 0.81 (3H, s, H-28); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 33.2 (C-1), 25.3 (C-2), 76.2 (C-3), 37.5 (C-4), 48.8 (C-5), 18.3 (C-6), 33.2 (C-7), 41.8 (C-8), 50.0 (C-9), 37.2 (C-10), 21.3 (C-11), 28.2 (C-12), 37.5 (C-13), 43.6 (C-14), 27.5 (C-15), 35.6 (C-16), 44.7 (C-17), 48.3 (C-18), 50.0 (C-19), 73.5 (C-20), 29.1 (C-21), 40.2 (C-22), 28.3 (C-23), 22.1 (C-24), 15.7 (C-25), 16.9 (C-26), 15.0 (C-27), 19.2 (C-28), 24.9 (C-29), 31.5 (C-30); FABMS *m/z* 467 $[M + Na]^+$, 449 $[M + Na - H_2O]^+$; HRFABMS *m/z* 467.3902 (calcd for $C_{30}H_{52}O_2Na$, 467.3865).

Grewin (2): white amorphous powder; $[\alpha]^{23}_D$ 0 (*c* 0.02 MeOH); IR (film) ν_{max} 3303, 2956, 2924, 2854, 1741, 1591, 1076 cm^{-1}; ^1H NMR (pyridine-d_5, 300 MHz) and ^{13}C NMR (pyridine-d_5, 75 MHz), see Table 1; positive ESIMS *m/z* 395 $[M + Na]^+$, 767 $[2M + Na]^+$; positive HRESIMS *m/z* 395.0814 (calcd for $C_{19}H_{16}O_8Na$, 395.0743).

Bilagrewin (3): yellow amorphous powder; $[\alpha]^{23}_D$ +3.3 (*c* 0.18 MeOH); IR (film) ν_{max} 3343, 2926, 2849, 1689, 1584, 1600, 1469, 1115 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz), see Table 1; positive ESIMS *m/z* 425 $[M + Na]^+$; positive HRESIMS *m/z* 425.1206 (calcd for $C_{21}H_{22}O_8Na$, 425.1212).

Acknowledgment. This project was supported by NIH Grant 1 U01-TW01015-01, administered by the Fogarty International Center as part of an International Cooperative Biodiversity Groups (ICBG) program, through funds from NIH, NSF, and Foreign Agricultural Service of the USDA. The permit for the collection and export of plant material for this study was granted by the Ministry of Agriculture and Rural Development, Hanoi, Vietnam, through a letter dated September 15, 1998, Ref. No. 3551/BNN/KHCN, and from the Cuc Phuong National Park, through a letter dated September 16, 1998. The authors are grateful to the Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy and the Research Resources Center, University of Illinois at Chicago, for support in the acquisition of the NMR and MS data.

References and Notes

(1) Greenberg, P. L.; Gordeuk, V.; Issaragrisil, S.; Siritanaratkul, N.; Fucharoen, S.; Ribeiro, R. C. *Hematology* **2001**, *2001*, 479−498.
(2) Soejarto D. D.; Gyllenhaal, C.; Regalado, J. C.; Pezzuto, J. M.; Fong, H. H. S.; Tan, G. T.; Hiep, N. T.; Xuan, L. T.; Binh, D. Q.; Hung, N. V.; Bich, T. Q.; Thin, N. N.; Loc, P. K.; Vu, B. M.; Southavong, B. H.; Sydara, K.; Bouamanivong, S.; Oneill, M. J.; Lewis, J.; Xie, X.; Dietzman, G. *Pharm. Biol.* **1999**, *37* (Suppl.), 100−113.
(3) Zhang, H. J.; Tamez, P. A.; Hoang, V. D.; Tan, G. T.; Hung, N. V.; Xuan, L. T.; Huang, L. M.; Cuong, N. M.; Tao, D. T.; Soejarto, D. D.; Fong, H. H. S.; Pezzuto, J. M. *J. Nat. Prod.* **2001**, *64*, 772−777.
(4) Zhang, H. J.; Qiu, S. X.; Tamez, P. A.; Hoang, V. D.; Tan, G. T.; Aydogmus, Z.; Hung, N. V.; Cuong, N. M.; Angerhofer, C.; Soejarto, D. D.; Pezzuto, J. M. Fong, H. H. S. *Pharm. Biol.* **2002**, *40*, 221−224.
(5) Zhang, H. J.; Tamez, P. A.; Aydogmus, Z.; Tan, G. T.; Saikawa, Y.; Hashimoto, K.; Nakata, M.; Hung, N. V.; Xuan, L. T.; Cuong, N. M.; Soejarto, D. D.; Pezzuto, J. M.; Fong, H. H. S. *Planta Med.* **2002**, *68*, 1088−1091.
(6) He, Z. D.; Ma, C. Y.; Zhang, H. J.; Tan, G. T.; Tamez, P.; Sydara, K.; Bouamanivong, S.; Southavong, B.; Soejarto, D. D.; Pezzuto, J. M.; Fong, H. H. S. *Chem. Biodivers.* **2005**, *2*, 1378−1386.
(7) Tanaka, R.; Matsunaga, S. *Phytochemistry* **1992**, *31*, 3535−3539.
(8) Yuruker, A.; Orjala, J.; Sticher, O.; Rali, T. *Phytochemistry* **1998**, *48*, 863−866.
(9) Hui, W. H.; Li, M. M. *Phytochemistry* **1977**, *16*, 111−112.
(10) Shamsuddin, T.; Rahman, W.; Khan, S. A.; Shamsuddin, K. M.; Kintzinger, J. P. *Phytochemistry* **1988**, *27*, 1908−1909.
(11) Ishikawa, T.; Fujimatu, E.; Kitajima, J. *Chem. Pharm. Bull.* **2002**, *50*, 1460−1466.
(12) Ishikawa, T.; Seki, M.; Nishigaya, K.; Miura, Y.; Seki, H.; Chen, I.; Ishii, H. *Chem. Pharm. Bull.* **1995**, *43*, 2014−2018.

(13) Ikuta, A.; Kamiya, K.; Satake, T.; Saiki, Y. *Phytochemistry* **1995**, *38*, 1203−1207.
(14) Sarngadharan, M. G.; Seshadri, T. R. *Tetrahedron* **1966**, *22*, 739−744.
(15) Li, S.; Lundquist, K.; Wallis, A. F. A. *Phytochemistry* **1998**, *49*, 2125−2128.
(16) Kumar, S.; Ray, A. B.; Konno, C.; Oshima, Y.; Hikino, H. *Phytochemistry* **1988**, *27*, 636−638.
(17) Matsushita, H.; Miyase, T.; Ueno, A. *Phytochemistry* **1991**, *30*, 2025−2027.
(18) Wu, L. J.; Zheng, J.; Jiang, B. H.; Shan, Z.; Shen, Y.; Liu, X. J.; Yan, S. M. *Acta Pharm. Sin.* **1999**, *34*, 294−296.
(19) Ma, C. Y.; Liu, W. K.; Che, C. T. *J. Nat. Prod*. **2002**, *65*, 206−209.

NP050313D